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Concerns:
Data package 2, submitted to NICEATM and ICCVAM for further evaluation of the LLNA and modifications of it

In 2005 the BG Institute for Occupational Safety and Health of the German Social Accident Insurance - BGIA (Berufsgenossenschaftliches Institut für Arbeitsschutz) initiated a meeting about skin sensitization, and the experiences so far with the Local Lymph Node Assay. Experts from different institutes (authority, academia, industry) in Germany discussed the data. There was a concern about the increase in positive results with LLNA compared to the years of experiences with guinea pig assays. This is also illustrated by the peer reviewed paper of Vohr and Ahr, 2005 [Ref. 2.1.]. During this meeting it was decided to compare the "standard" - radioactive - LLNA with a non-radioactive modification, i.e. cell counting, with 13 related compounds (epoxy resin components); most of which are classified as skin sensitizers based on guinea pig data. HCA was chosen as positive control. In accordance with the exemplary described method in OECD 429 mouse strain CBA was used for this study. For further information about the compounds and protocol see also Ref 2.2. and 2.3., and Table 1 below. Although both PP presentations are in German the main messages are clear and self-explanatory.

One of the goals was to correlate stimulation indices of both methods as well as cut-off concentrations evaluated by them, i.e. the effective or estimated concentrations of test items exceeding the cut-off lines defined for both methods. These EC values correspond to EC3 for the radioactive labeling or EC1.5 for the cell counting as also described previously [Ref. 2.4.].

Another aim of this study was to classify the test substances according to their potency to induce cell proliferation in the draining lymph nodes. This classification was based on the ECETOC

criteria described before [Ref. 2.5.]. Due to the fact that applications of moderate to strong irritants could result in false positive reactions ear weight was measured in addition to balance the influence of such non-specific cell activation. It has to be mentioned, however, that here skin reactions were measured three days after the last application (on day 6) while the "acute" skin reaction has reasonably to be measured one day after the last application on day 4. In case of 6 days protocols this parameter could be determined by measuring ears swelling at day 4 which was unfortunately not possible during this study. However, this has no influence on the overall assessment of the results, esp. on the comparison of estimated concentrations and stimulation indices.

Following 13 related test substances have been chosen for the comparison (Table 1):
Acetone was used as vehicle to reach acceptable solubility for all test items. Therefore, the positive control HCA was also tested in acetone.

Bisphenol A, resin, Bakelite EPR 164 (CAS-Nr. 25068-38-6)
Bisphenol A, resin, distilled, Bakelite EPR 162 (CAS-Nr.1675-54-3)
Bisphenol F, resin, Bakelite EPR 161 (CAS-Nr. 9003-36-5)

1,6-Hexanediol Diglycidyl Ether (CAS-Nr. 16096-31-4)
P-Tertbutylphenyl Glycidyl Ether (CAS-Nr. 3101-60-8)
Trimethylolpropane triglycidyl ether (CAS-Nr. 3454-29-3)
Dodecyl/tetradecyl glycidyl ether (CAS-Nr. 68609-97-2)

m-Xylylenediamine (CAS-Nr. 1477-55-0)
3-Aminomethyl-3,5,5-trimethylcyclohexylamine (CAS-Nr. 2855-13-2)
Bis(3-aminopropyl)amine (CAS-Nr. 56-18-8)
2,2,4(2,4,4)-Trimethyl-1,6-hexanediamine (CAS-Nr. 25620-58-0)
N-(2-Hydroxyethyl)ethylenediamine (CAS-Nr. 111-41-1)
1,2-Diaminocyclohexane (CAS-Nr. 694-83-7)

All the studies have been conducted at BASF AG, Ludwigshafen, Germany, under full GLP compliance. Data were presented by the study director, Dr. A.O. Gamer, and discussed in a similar panel as before.

Conclusions:

--- There was an extremely good correlation between stimulation indices obtained by radioactive labeling and non-radioactive cell counting [see also Fig. 1 below and Ref. 2.6.].

--- Therefore, the effective concentrations calculated are very similar for both endpoints [see also Table 2 below and Ref. 2.6.].

--- The vehicle (acetone) may have an impact in the relatively low effective doses (i.e. relative high potency) determined for the test substances. This may easily be recognized by the results obtained with HCA diluted in acetone alone or acetone:olive oil (AOO 4:1).

--- Taken the irritant potential also into account will improve the assessment of the overall sensitizing potency. However, optimal time point for the determination of acute skin reaction is one day after last application, i.e. day 4 in standard protocol.

Figure 1: Comparison of lymph node cell count and ^{3}H-thymidine incorporation taken from the Report by AO Gamer and R Landsiedel [Ref 2.6.]

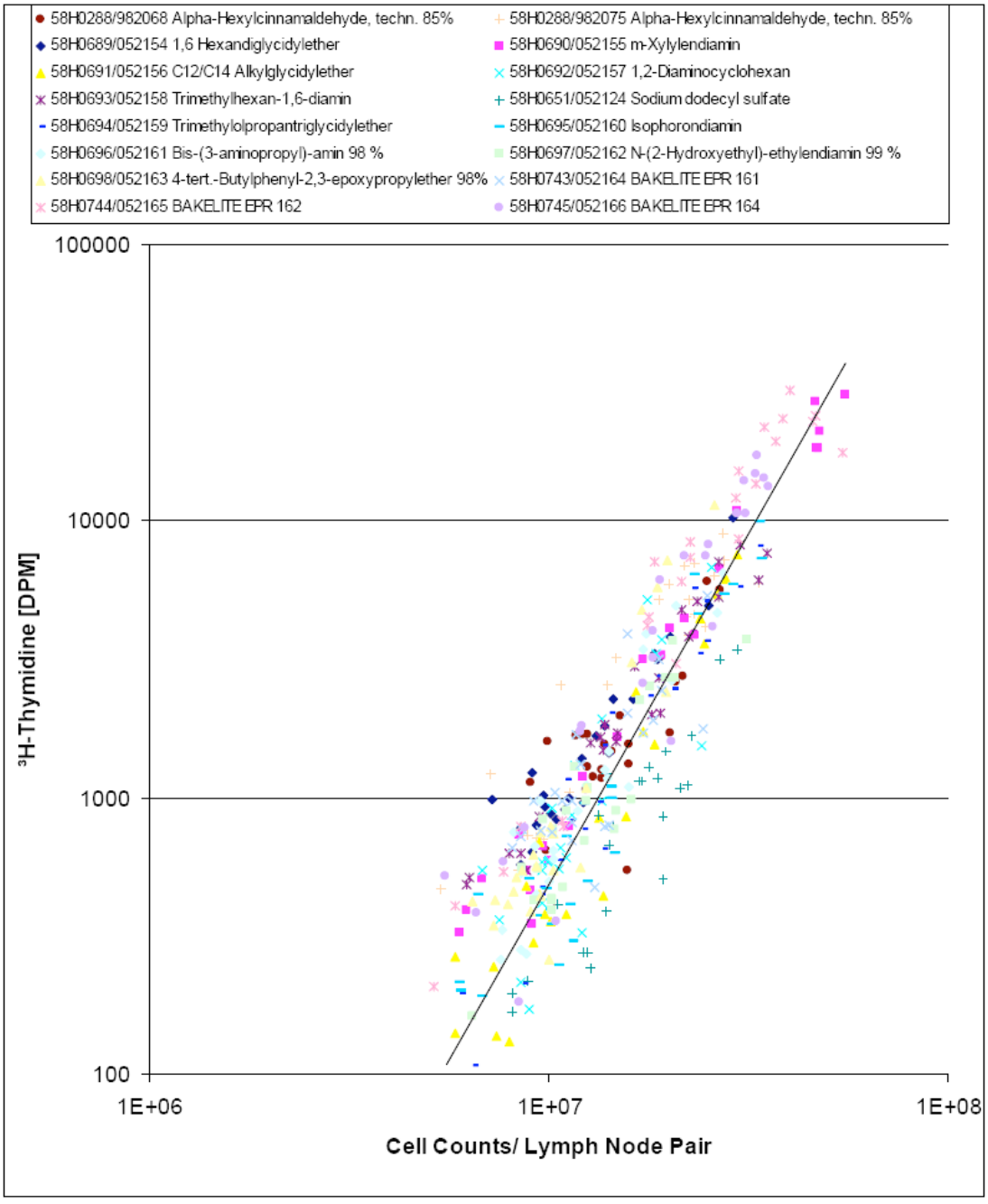

Table 2: Tested concentrations and "Estimated Concentrations[1]" of skin sensitising threshold of epoxy resin components from Ref. 2.6.

Substance name	Concentrations tested	Results			
	% (w/w)	EC3	EC1.5	EC3lg	EC1.5lg
1,6-Bis(2,3-epoxypropoxy)hexane	3, 1, 0.3	1.9	1.7	1.6	1.5
m-Phenylenebis(methylamine)	3, 1, 0.3	0.4	0.4	0.4	0.3
Oxirane, mono((C12-14-alkyloxy)methyl)derivs	3, 1, 0.3	0.6	0.7	0.5	0.6
1,2-Diaminocyclohexane	1, 0.3, 0.1	0.4	0.6	0.4	0.5
Trimethylhexamethylene diamine	10, 3, 1	1.9	0.5	1.7	0.8
1-(2,3-Epoxypropoxy)-2,2-bis[(2,3-epoxypropoxy)methyl]butane	10, 3, 1	1.4	1.7	1.3	1.4
3-Aminomethyl-3,5,5-trimethylcyclohexylamine	3, 1, 0.3	1.0	1.2	1.0	1.1
Dipropylene triamine 98%	3, 1, 0.3	0.9	1.0	0.8	1.0
N-(2-Hydroxyethyl)-ethylendiamine 99%	30, 10, 3	15.2	14.4	13.3	12.7
p-tert-Butylphenyl 1-(2,3-epoxy)propyl ether 98%	1, 0.3, 0.1	0.4	0.5	0.3	0.4
Bakelite EPR 161	1, 0.3, 0.1	0.7	0.6	0.6	0.5
Bakelite EPR 162	3, 1, 0.3	-	-	0.2	0.1
Bakelite EPR 164	3, 1, 0.3	0.1	-	0.2	0.1
α-Hexyl cinnamic aldehyde/AOO	10, 5, 2.5	10.5	6.9	10.7	6.5
α-Hexyl cinnamic aldehyde/Acetone	30, 10, 3	-	0.1	1.2	1.8

- no meaningful calculation possible

[1] Estimated concentration that leads to the respective stimulation index
EC was estimated by linear regression
EClg was estimated by linear regression using a log transformation of the concentration

Kind regards,

H.-W. Vohr

References:

2.1. Vohr HW and Ahr HJ.
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Archive of Toxicol. 79, 721-8, 2005

2.2. PP file about the test compounds (epoxy resin components) presented at the kick-off meeting by K. Kefenbaum from BGIA

2.3. PP file about the pros and cons of the Local Lymph Node Assay (LLNA) and test compounds presented at the kick-off meeting by A.O. Gamer from BASF

2.4. Ehling G, Hecht M, Heusener A, Huesler J, Gamer AO, v. Loveren, H., Maurer Th, Riecke K, Ullmann L, Ulrich P, Vandebriel R, Vohr H-W
An European Inter-Laboratory Validation of Alternative Endpoints of the Murine Loacl Lymph Node Assay. 2nd ROUND.
Toxicology, 212, 69-79, 2005

2.5 Basketter, D.A., Butler, M. Gamer, A., Garruige, J.-L., Gerberick, G.F., Kimber, I., Newsome, C., Steiling, W. and Vohr, H.-W.
Contact Sensitisation: Classification According to Potency.
Technical Report No. 78, ECETOC, Brussels (2003).

2.6. Summary report of the GLP studies with all test compounds of the validation described by AO Gamer and R Landsiedel, BASF AG, Germany